EXHIBIT 3

Original Article

Results of the ACTION-Galactosemia Kids Study to Evaluate the Effects of Govorestat in Pediatric Patients with Classic Galactosemia

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Abstract

To evaluate the pharmacodynamic effects and clinical outcomes of orally administered once-daily govorestat (AT-007), a central nervous system penetrant aldose reductase inhibitor, the double-blind placebo-controlled ACTION-Galactosemia Kids study (NCT04902781) randomly assigned 47 participants (2–17 years old) with Classic Galactosemia to 18 months of govorestat or placebo (2:1) treatment. Mean change in galactitol was compared between the treatment groups at each post-baseline timepoint using a t-test, with a mixed model for repeated measures (MMRM) analysis as a sensitivity analysis. Changes from baseline in clinical outcomes were compared between treatment groups also using a t-test with two different MMRM models as sensitivity models, one including baseline clinical outcome score. The pharmacodynamic effect of govorestat was assessed by correlating galactitol level at 3 months with change from baseline in clinical measures at 18 months using a Pearson correlation. Govorestat treatment resulted in a rapid and sustained reduction in plasma galactitol. Govorestat treatment stabilized or improved clinical measures of behavior, daily living skills, adaptive skills, cognition, tremor, and fine motor skills, which declined over time in the placebo group. Govorestat treatment did not demonstrate a benefit compared with placebo on speech outcomes or gross motor skills, which improved in both treatment groups over 18 months. Govorestat was safe and well tolerated, with adverse events well balanced between the active and placebo groups. Aldose reductase inhibition with govorestat represents a potential opportunity to lower galactitol and improve clinical outcomes in children with Classic Galactosemia.

Keywords

aldose reductase, AT-007, Classic Galactosemia, govorestat, pediatric

Introduction

Classic Galactosemia is a rare autosomal recessive metabolic disorder, which affects the body's ability to metabolize the simple sugar galactose. Patients with Classic Galactosemia have mutations in the gene encoding the galactose-1-phosphate uridyltransferase (GALT) enzyme, resulting in <1% GALT enzyme activity. As a result of this severe enzyme deficiency, patients are unable to metabolize galactose properly. Galactose is found in foods, primarily dairy products and breast milk, where lactose is metabolized to galactose and glucose. Additionally, fruits, vegetables, legumes, and other non-dairy products contain low levels of galactose. The current standard of care is universal newborn screening to identify infants at birth with Classic Galactosemia, and immediate long-term dietary restriction of foods containing high levels of galactose.[1] This has been largely successful in preventing fatalities and acute complications due to galactose exposure in the perinatal period. However, the body produces galactose endogenously through de novo synthesis, which leads to high levels of galactose and galactose metabolites

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despite dietary restriction, and results in long-term neurological complications in patients with Classic Galactosemia.[2–4]

Galactose is normally metabolized through the Le Loir pathway, where two critical enzymes are involved. Galactokinase (GALK) metabolizes galactose to galactose-1-phosphate (Gal-1p). Gal-1p is then metabolized to glucose by GALT. When patients are deficient in either the GALK enzyme or the GALT enzyme, galactose levels accumulate in the body, and galactose becomes an aberrant substrate for the enzyme aldose reductase. Aldose reductase converts galactose to an aberrant, toxic metabolite called galactitol, which is an end product that does not form in healthy individuals. For many years, a biochemical debate ensued as to which galactose metabolite was responsible for long-term complications in galactosemia. Recent work has demonstrated that galactitol is the toxic metabolite responsible for long-term complications in galactosemia and suggested that galactitol reduction through inhibition of the enzyme aldose reductase may be a beneficial clinical approach to treating patients with Classic Galactosemia.[5,6]

The clinical picture of Classic Galactosemia evolves over the lifetime of the patient. After resolution of any acute symptoms during the newborn period, infants often appear normal, and developmentally achieve milestones similar to their peers. However, despite strict dietary restriction of galactose, patients develop significant long-term neurological and behavioral complications, which often first appear in early school age children and worsen over time. These symptoms include behavioral abnormalities, cognitive impairment, tremor, issues with fine and gross motor skills, and speech problems. Females develop primary ovarian insufficiency.[3,4] Neurological and behavioral symptoms impact patients' ability to perform activities of daily living (ADLs). As adults, patients with Classic Galactosemia are primarily unable to live alone and must depend on a caregiver.[7] Therefore, there is a need to develop treatments for Classic Galactosemia beyond dietary restriction of galactose to prevent long-term neurological and behavioral complications.

The objective the study described in the present report was to investigate efficacy, pharmacology, and safety of govorestat, a central nervous system penetrant aldose reductase inhibitor in pediatric patients with Classic Galactosemia.

Methods

Study Design

The ACTION-Galactosemia Kids study (ClinicalTrials.gov identifier: NCT04902781) protocol and informed consent forms (ICFs) were reviewed and approved by the Institutional Review Boards (IRBs) for each of the three study sites: ADVARRA for the Rare Disease Research Center (Atlanta, GA) and for the Michigan Medicine University of Michigan (Ann Arbor, MI); WCG IRB for Colorado Children's Hospital (Denver, CO). The parents and/or legally authorized representatives of all study participants provided written, informed consent, and all participants able to do so assented to being in the study. Furthermore, this study was conducted in accordance with the ethical principles of International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and all applicable local Good Clinical Practices (GCPs) and regulations, including the archiving of essential documents. All United States of America Food and Drug Administration (FDA) regulatory requirements were followed, in particular, those affording greater protection to the safety of vulnerable pediatric participants.

The ACTION-Galactosemia Kids study was designed to evaluate the safety, pharmacodynamic efficacy, and clinical outcomes efficacy of govorestat, a once-daily oral aldose reductase inhibitor, in children aged 2 to 17 years with Classic Galactosemia. The hypothesis was that treatment with govorestat should decrease levels of the toxic galactose metabolite, galactitol, in blood and tissues, and improve neurological and behavioral outcomes in children with Classic Galactosemia. The study was a randomized, double-blind, placebo-controlled study with a 2:1 randomization of active drug to placebo that was conducted at three academic centers in the United States. The first participant was enrolled at the Rare Disease Research Center in Atlanta, Colorado Children's Hospital in Denver, and the University of Michigan in Ann Arbor on March 20, 2021; July 19, 2021; and May 12, 2021, respectively, and the last participant at each respective center on July 22, 2021; July 19, 2021; and July 14, 2021. The study concluded on March 24, 2023.

An independent biostatistician generated the randomization scheme. Clinical personnel remained blinded to study treatment. Children were stratified across three age groups (2-6; 7–12; 13–17 years old) to ensure enrollment was evenly distributed across the large age range of 2–17 years; however, results were evaluated together across all ages in the active versus placebo groups. The study included a dose-escalation period to determine the appropriate dose exposure in children, followed by a pharmacodynamic evaluation of galactitol reduction, and clinical outcome assessments every 6 months (additional study design and study conduct information included in the Supplemental Methods). Because Classic Galactosemia is a rare disease, which had not been

previously studied in any long-term longitudinal natural history studies, the conceptual design was to measure clinical outcome changes over a broad range of symptoms, including behavior, cognition, fine motor skills, gross motor skills, tremor, and speech outcomes, and to evaluate the sum of change across these domains in the active versus placebo groups, using a statistical methodology called the Wei-Lachin Global Statistical Test. The expectation was that the placebo group should decline over time; whereas the govorestat group should stabilize or improve. Because of the paucity of prior data to determine how long it would take to achieve clinical benefit, and the ethical considerations regarding treating children with placebo, a firewalled Data Monitoring Committee (DMC) was designated to evaluate the unblinded clinical outcomes and safety data every 6 months, and to inform the Sponsor to unblind the study once clinical benefit with govorestat was demonstrated, or if futility was reached.

Enrollment Criteria

Children 2–17 years old with Classic Galactosemia were recruited to the study. Participants were required to have <1% GALT enzyme activity (assessed by Mayo Clinic assay) and genetic confirmation of Classic Galactosemia (performed by GeneDx). Participants had to be compliant with a galactose-restricted diet and willing to remain compliant over the course of the study. Full inclusion and exclusion criteria can be found in the Supplementary Methods.

Clinical Outcome Assessments

Behavioral outcomes were assessed using the Behavioral Assessment Scales for Children 3 Parent-Reported Scales (BASC-3 PRS) and the Vineland-3 assessor-mediated interview. Originally, the study contemplated Vineland as the primary behavioral assessment scale to be included in the primary endpoint with BASC included as a secondary endpoint. However, it was determined through a blinded audit of Vineland during the trial that the interviewers were not performing the assessments properly, despite training and certification. Therefore, the primary endpoint was adjusted to include BASC for behavioral assessments, and Vineland became an exploratory outcome measure. For BASC, caregivers completed a parent-reported questionnaire directly on a paper or electronic form as directed by the developer's manual. Three age-designated questionnaires were used: BASC Preschool for children aged 2 to 5 years; BASC Child for children aged 6 to 11 years; and BASC Adolescent for children aged 12 to 17 years. If a child "aged out" of the questionnaire that they started the study with (i.e., started the study in one questionnaire age group and transitioned to the next older questionnaire age group), change from one test to another could not be directly compared. The primary analysis methodology excluded these children from the BASC change over time calculations, and an imputation methodology was used to impute changes across the two different questionnaires (see Statistical Methods section). Cognition was assessed using the National Institutes of Health (NIH) Toolbox Cognition Battery version 2 (NIH-CB) administered on an iPAD or computer for all children aged 3 years and older. Motor skills were assessed using NIH Motor Battery with fine motor skills assessed using the 9-Hole-Pegboard Test (9HPT) and gross motor skills assessed using the standing balance test in children aged 3 years and older. All NIH Toolbox tests were administered and scored using the developer's manual. Speech was assessed using the Oral and Written Language Skills test (OWLS-II), which is comprised of two units, Oral Expression (OE) and Listening Comprehension (LC), administered to children aged 3 years and older. For standardized tests (OWLS-II, BASC-3, and NIH Toolbox), age-standardized scores (standardized to age-matched normal controls) were used for primary analysis, and raw scores (raw numerical results) were used in exploratory and supportive analyses. Tremor was assessed using the Archimedes Spiral Drawing test scored (scale of 0–4) by a blinded panel of neurologists in children aged 5 years and older.

Parents completed Global Impression of Severity (GIA) questionnaires at baseline and every 6 months, and exit interviews were performed at the point of study completion (prior to unblinding and by an independent third party) to provide information on clinical meaningfulness of any changes over time.

Pharmacology and Biomarker Assessments

Govorestat, galactitol, and galactose plasma levels, and Gal-1p whole blood levels were determined on Days 1 and 5 for participants in the dose-escalation cohort via serial pharmacokinetic (PK) sampling at time 0, 2, 4, 8, 12, and 24 h post-dosing. For the main portion of the study, PK assessments were performed on Day 1, Day 30, Day 60, and Day 90 of the study using serial PK assessments. At Months 6, 12, and 18, limited PK and pharmacodynamic (PD) samples were drawn at predose and at 8 h post-dose to minimize blood draws for participants.

Govorestat, galactitol, and galactose were analyzed in human plasma using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) validated methods. Gal-1p was analyzed in human whole blood using a qualified LC-MS/MS method. All bioanalytical and pharmacological assays were developed at ICON Labs, Whitesboro, NY, as previously described.[8]

Safety Assessments

Safety assessments were performed on the same days as PK/PD assessments and consisted of vital signs, electrocardiograms (ECGs), and standard safety labs including hematology, serum chemistry, and coagulation along with urinalysis, urine albumin to creatinine ratio (UACR), urine protein to creatinine ratio (UPCR), and urine markers of kidney injury (clusterin [CLU], cystatin-C [CysC], kidney injury molecule-1 [KIM-1], N-acetyl-beta-d-glucosaminidase [NAG], neutrophil gelatinase-associated lipocalin [NGAL], and osteopontin [OPN]).

Adverse events (AEs) were monitored throughout the study. All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 24.0. A treatment-emergent adverse event (TEAE) was defined as an AE with onset on or after the date/time of the first dose of study drug. TEAEs were tabulated by MedDRA System Organ Class (SOC) and Preferred Term (PT).

Study Drug Administration

Govorestat drug product oral suspension or matching placebo were administered once daily via oral syringe in the morning following an overnight fast. Treatment compliance and dietary adherence were monitored via participant diaries.

Statistical Methods

The PK and PD analyses of govorestat, galactitol, and galactose in plasma and Gal-1p in whole blood were done using noncompartmental methods in Phoenix WinNonlin (Version 6.3 or higher, Pharsight Corporation) and/or SAS (Version 9.4 or higher, SAS Institute Inc.).

All other statistical analyses were done using SAS (Version 9.4 or higher, SAS Institute Inc.).

Mean change in galactitol was compared between the govorestat and placebo groups at each post-baseline timepoint using a t-test with a sensitivity model using a mixed model for repeated measures (MMRM) analysis.

Each clinical efficacy endpoint was analyzed by comparing the change from baseline to 6, 12, and 18 months between treatment groups using a t-test. Degrees of freedom were approximated using the Satterthwaite method with no imputation for missing values. A sensitivity analysis using an MMRM was conducted using change from baseline to 18 months. The limited model included fixed effects for treatment, visit, and treatment by visit interaction, and a second model additionally included fixed effects for baseline covariate value and baseline by visit interaction. Both models used an unstructured covariance matrix for the random effects.

The pharmacodynamic effect of govorestat was assessed by using a Pearson correlation for each outcome to determine the association between Month 3 galactitol levels and the change from baseline to Month 18 for the clinical measure of interest.

A subset of participants (N = 9) became too old for the version of the BASC questionnaire that they were originally tested on and were subsequently tested on the older (age appropriate) version of the BASC. To account for this difference in BASC questionnaire administration, scores were imputed at later ages for these participants. First, scores from BASC versions other than the first one they were administered were set to missing. Then, multiple imputation was used to generate a set of possible scores for the missing values, and the median of the imputed scores for each value was used as the new value. Scores were imputed using a model with baseline BASC score for that item, and the current value of all other BASC items as well as age, visit, and treatment group. The imputation was done using SAS PROC MI.

Results

Demographic and Baseline Characteristics

Forty-seven children with Classic Galactosemia were enrolled in the study, which ran from March 20, 2021 through March 24, 2023. Of 16 children randomly assigned to placebo, one discontinued the study due to a TEAE and another was withdrawn by a parent or legal guardian. In the govorestat group (n = 31), two children discontinued due to TEAEs and three were withdrawn by a parent/guardian (Figure 1). There were no other discontinuations or withdrawals from the study; no participants were lost to follow-up; and there were no deaths.

Baseline characteristics (Table 1) were comparable between treatment groups and were reflective generally of the overall Classic Galactosemia population. All children enrolled in the study had <1% GALT enzyme activity, elevated plasma levels of galactose and galactitol, and elevated erythrocyte levels of Gal-1p. The mean age of study participants was 9.2 years; enrollment was balanced between males and females. All but one participant were white and non-Hispanic, reflecting the known prevalence of Classic Galactosemia amongst those of primarily Caucasian descent. In the study population, 40.4% of participants were homozygous for the most comment GALT genetic mutation (Q188R); 42.6% of participants were compound heterozygous with one allele affected by the Q188R mutation and the other allele affected by a unique mutation. The remainder (17.0%) of the study population had unique mutations on both alleles.

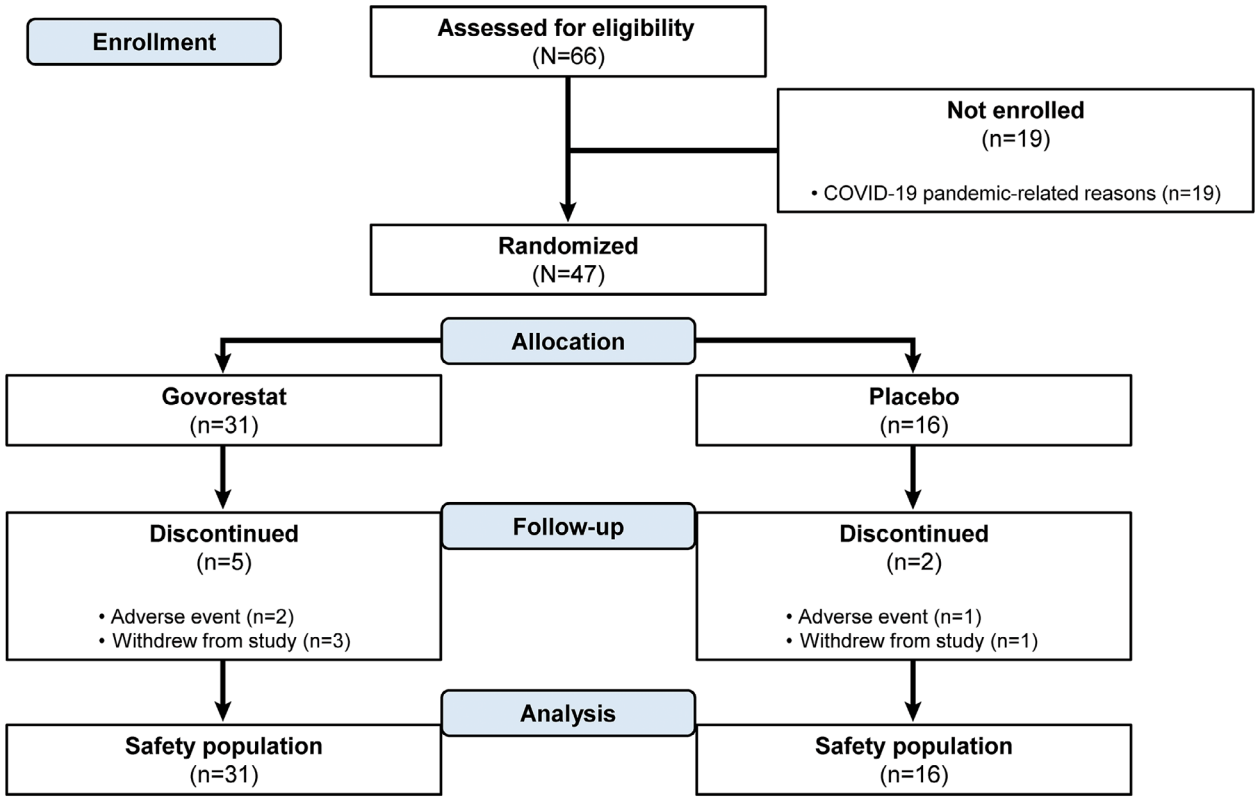

Figure 1. Participant disposition.

Table 1. Participant Demographics and Biochemical Characteristics

Characteristic	Placebo (N = 16)	Govorestat (N = 31)
Mean age, years (SD)	9.7 (4.44)	8.9 (4.21)
Sex (% male)	50.0%	48.4%
Race (% white)	100%	96.8%
Mean BMI (SD)	17.42 (4.06)	16.38 (1.90)
Mean GALT enzyme activity, mmol/h/mg (SD)	0.02 (0.04)	0.01 (0.03)
Mean plasma galactose, ng/mL (SD)	631 (66.1)	593 (129)
Mean plasma galactitol, ng/mL (SD)	2202 (214)	1807 (459)
Mean erythrocyte Gal-1p, ng/mL (SD)	16174 (3781)	15379 (6514)

BMI, body mass index; Gal-1p, galactose-1-phosphate; GALT, galactose-1-phosphate uridyltransferase; SD, standard deviation.

Pharmacokinetics/Dose Determination

Initially in the dose-escalation period of the study, the govorestat dose was based on age and demonstrated a near-linear dose-dependent increase in maximal concentration (C_{max}) and area under the curve (AUC). However, a review and modeling by an unblinded pharmacologist revealed that body weight rather than age was a better determinant of dose exposure. Therefore, dosing was converted to weight-based dosing for the long-term clinical outcomes portion of the study with children weighing under 20 kg dosed at 30 mg/kg; children weighing 20 to 40 kg dosed at 20 mg/kg; and children weighing over 40 kg dosed at 15 mg/kg. Dosing based on weight ranges is observed for many drugs in pediatric populations with smaller children having less surface area for exposure and requiring a higher dose on a mg/kg basis. At the doses outlined above, participants achieved a uniform exposure with a C_{max} of 44.01 µg/mL (geometric percent coefficient of variation [GeoCV%] of 45.28) and AUC_{tau} of 381.89 µg*h/mL (GeoCV% of 45.61) with a time to maximal concentration (T_{max}) of 4 h after dosing.

Summary statistics for pharmacokinetics on Day 30, 60 and 90 is provided in Table S2. No measurement of drug PK occurred after Day 90.

Clinical Outcomes

Initially, the primary clinical endpoint of the study was designed as a composite sum of change across behavior (with key subunits being ADLs and behavioral symptoms), cognition, fine motor skills (9HPT), gross motor skills (standing balance), tremor, and speech (OWLS-II-OE and OWLS-II-LC). However, due to feedback from regulatory agencies (i.e., FDA) over the course of the study, the primary endpoint was modified to include BASC ADLs and BASC behavioral symptoms (Behavioral Symptoms Index [BSI]) as well as the two speech components (OWLS-II-OE and OWLS-II-LC). The NIH-CB and 9HPT were included in sensitivity

analyses to the primary endpoint. Standing balance and tremor became secondary endpoints along with the additional behavioral measures included in the BASC.

At 18 months of treatment, the DMC advised the Sponsor to consider unblinding the study, due to benefit of treatment for the govorestat group and ethical issues regarding continuing a placebo group in light of clinical benefit in the govorestat group and clinical decline in the placebo group. While the primary endpoint had not reached statistical significance, several of the key secondary endpoints were statistically significant and demonstrated substantial clinical benefit of govorestat treatment at 18 months. Additionally, it was clear to the DMC that the two speech components of the primary endpoint were somehow confounded as speech performance improved over time in both the active and placebo groups, instead of the expected decline in the placebo group. Therefore, it did not appear to the DMC that decisions regarding clinical benefit should be based solely on the primary endpoint.

Treatment effect of govorestat over the components of the primary endpoint (BASC-ADL, BASC-BSI, OWLS-OE, and OWLS-LC), the sensitivity analysis components (NIH-CB and 9HPT), and secondary clinical endpoints (BASC additional behavioral components, standing balance, and tremor) are shown in forest plots in Figure 2a,b. Govorestat demonstrated a treatment benefit on BASC endpoints including ADL, BSI, Adaptive Skills Index (ASI), adaptability, withdrawal, attention deficit hyperactivity disorder (ADHD) probability index, and social skills; cognition assessed by NIH-CB, fine motor skills assessed by 9HPT, and tremor assessed by the Archimedes Spiral Drawing test. Govorestat did not demonstrate a treatment benefit on speech and language (OWLS-OE and OWLS-LC) or standing balance (Figure 2b).

Therefore, the primary endpoint demonstrated separation over time between the govorestat and placebo groups as shown in Figure 2c but did not achieve statistical significance defined as a P-value $\leq .05$ at 18 months. The sensitivity analysis to the primary endpoint which included cognition did achieve statistical significance with a P-value of .03 at 18 months (Figure 2c). Additional statistical methodologies, such as imputation and mixed models, included in supportive analyses provided similar results.

An analysis of change over time on clinical endpoints demonstrated that except for speech and standing balance, the placebo group worsened over 18 months on standardized tests, while the govorestat group either stabilized or improved (Figure 3 and Table 2). Raw scores and BASC scores with imputation for participants who aged out of their initial test form are shown in Figure S1. With regard to speech (OWLS-OE and OWLS-LC) and gross motor skills (standing balance), both the placebo and active groups improved over time. This was hypothesized to be due to confounding use of speech therapy and occupational therapy in these groups, which was not controlled over the course of the study. BASC endpoints are shown as age-standardized T scores (scale of 0–50 where each standard deviation is 10 points); NIH Toolbox outcomes of cognition, 9HPT, standing balance, and OWLS endpoints are shown as age-standardized scores (scale of 0–100 where each standard deviation is 15 points); tremor is on its own severity scale of 0–4, where each 1-point change is an increasing magnitude of tremor.

Pharmacodynamic Biomarker Effects

Following govorestat administration, plasma galactitol levels decreased rapidly, starting on the first day of treatment. At Month 3, galactitol was reduced by 40.19% in the govorestat group, which was highly statistically significant compared to the placebo group ($P < .001$). The decrease in galactitol level was sustained over the 18-month treatment period in the govorestat group; whereas, the galactitol level in the placebo group did not change over time (Figure S2). The decrease in galactitol level was not associated with any compensatory increase in the circulating levels of galactose or Gal-1p.

Because the hypothesis of the study was that govorestat treatment should result in clinical outcomes benefit by reducing levels of toxic galactitol, a correlation analysis (Pearson correlation) was performed evaluating the correlation of galactitol level at 3 months (an early timepoint before clinical outcomes were assessed) with change in clinical outcomes at 18 months (Figure 4). For the clinical outcome measures that demonstrated treatment effects, a strong correlation was apparent between early galactitol level reduction and later improvement in clinical outcomes. Participants with lower galactitol levels had more favorable clinical outcomes compared with those who had higher galactitol levels. Results were similar when imputation was applied to the participants who switched BASC tests during the study (Figure 4).

Clinical Meaningfulness of Change

Change in Caregiver GIS was evaluated for govorestat as compared to placebo to determine whether the changes seen in individual clinical outcomes resulted in an overall picture of clinical change that was detectable to caregivers. Caregivers assessed the clinical severity of their child as 0 (normal; age appropriate); 1 (mildly impaired); 2 (moderately impaired); 3 (markedly impaired); or 4 (severely impaired). Figure 5 shows the change in GIS, demonstrating improvement in the govorestat group as compared to the placebo group.

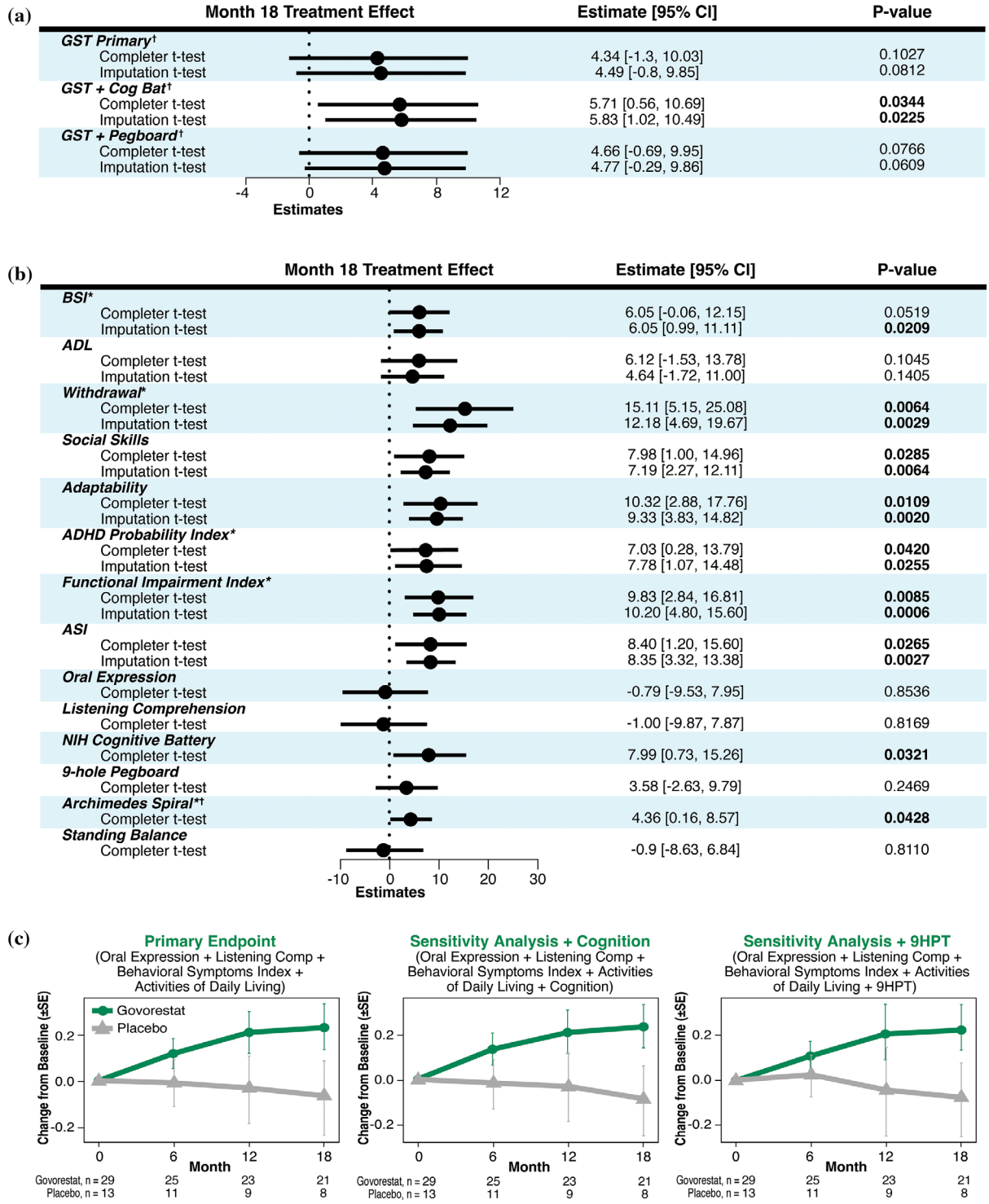

Figure 2. Forest plots of the composite primary endpoint and components of this primary endpoint with change in primary endpoint over time. (a) Forest plot displaying the primary endpoint global statistical test and sensitivity analyses to the primary endpoint including cognition and 9-Hole-Pegboard Test (9HPT). (b) Forest plot displaying individual components of the primary endpoint (Behavior Assessment System for Children Activities of Daily Living [BASC-ADL], Behavior Assessment System for Children Behavioral Symptoms Index [BASC-BSI], Oral and Written Language Scales-Oral Expression [OWLS-OE], and Oral and Written Language Scales-Listening Comprehension [OWLS-LC]); components of the sensitivity analyses to the primary endpoint (National Institute of Health Cognition Battery [NIH-CB] and NIH Motor Battery 9HPT); and key secondary endpoints (BASC social skills, withdrawal, adaptability, Adaptive Skills Index [ASI], attention deficit hyperactivity disorder [ADHD] probability index, and functional impairment index; NIH Motor Battery standing balance test; and tremor). Brackets show 95% confidence intervals (CI). *Variables were reversed so that higher scores represented improvement. †Archimedes Spiral Drawing test for tremor was multiplied by 10 to match the scales of the other tests. (c) Change over time in z-scores for the composite primary endpoint and sensitivity analyses including cognition and fine motor skills from baseline to 6, 12, and 18 months.

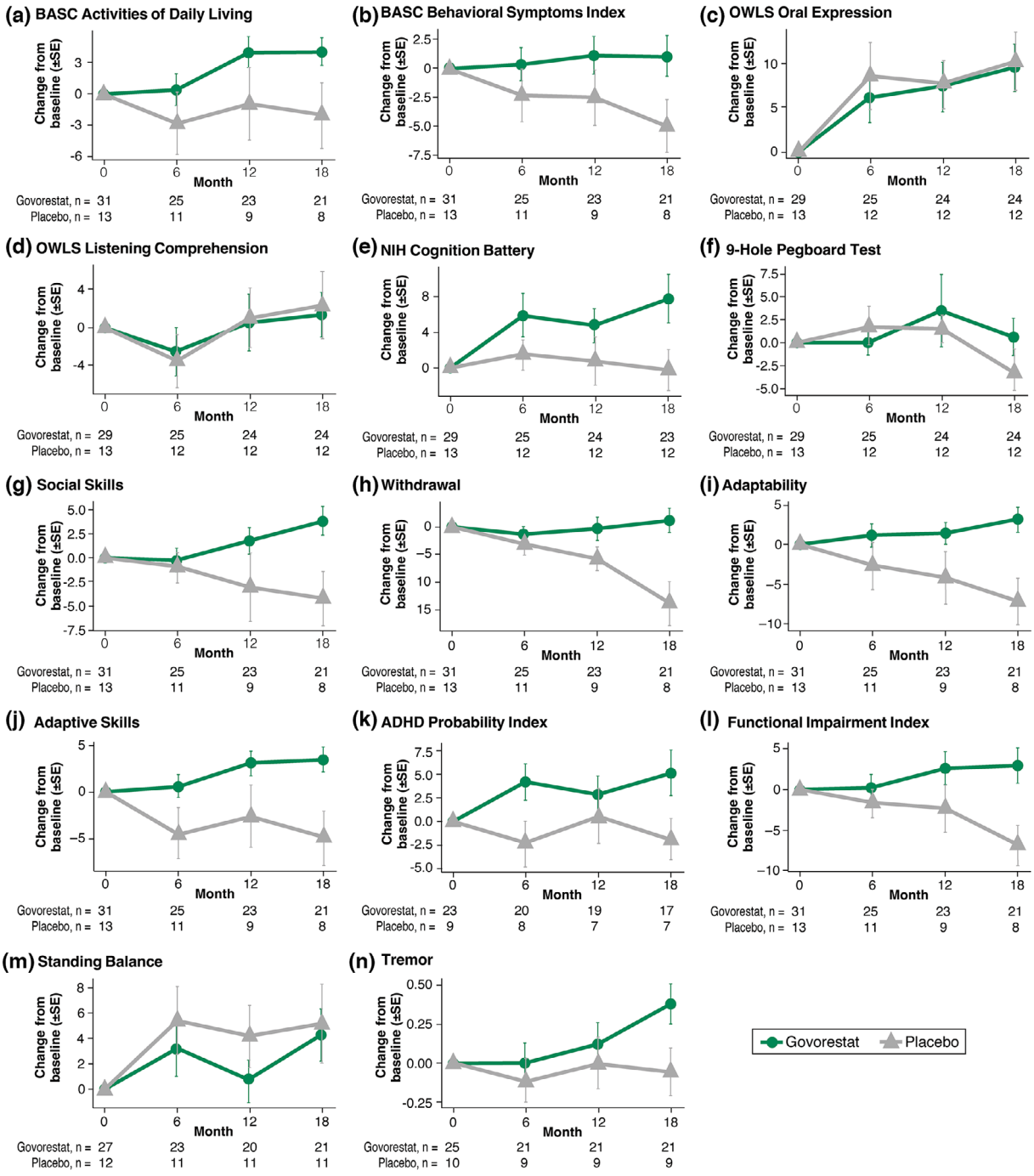

Figure 3. Mean change from baseline to 6, 12, and 18 months for the govorestat and placebo treatment groups on age-standardized rests. (a) Behavior Assessment System for Children Activities of Daily Living (BASC-ADL). (b) Behavior Assessment System for Children Behavioral Symptoms Index (BASC-BSI). (c) Oral and Written Language Scales-Oral Expression (OWLS-OE). (d) Oral and Written Language Scales-Listening Comprehension (OWLS-LC). (e) National Institute of Health Cognition Batter (NIH-CB). (f) 9-Hole-Pegboard Test (9HPT). (g) BASC social skills. (h) BASC withdrawal. (i) BASC adaptability. (j) BASC adaptive skills. (k) BASC attention deficit hyperactivity Disorder (ADHD) probability index. (l) BASC functional impairment index. (m) NIH standing balance test. (n) Tremor (not age standardized). For all graphs, error bars show standard error (SE).

Additionally, qualitative exit interviews were performed prior to study treatment unblinding, asking caregivers whether they saw changes (worsening, no change, or improvement) in their child over the course of the study, and whether this change was clinically meaningful to them (Figure 5). In all cases, the changes were clinically meaningful, demonstrating that caregivers could detect the clinical changes on a day-to-day basis, and that worsening, stabilization, or improvement were meaningful to them. This data was used in a determination of

Table 2. Mean Change from Baseline to Month 18 in Clinical Outcomes in the Govorestat and Placebo Treatment Groups

Clinical Outcome	Mean Change Govorestat (SE)	Mean Change Placebo (SE)	Mean Treatment Difference Govorestat/Placebo (SE)	*P*-value
Behavioral symptoms	Improvement 1.05 points (1.76)	Worsening 5.00 points (2.28)	6.05 points (2.88)	.052
Activities of daily living	Improvement 4.00 points (1.33)	Worsening 2.13 points (3.15)	6.13 points (3.41)	.105
Adaptive Skills Index	Improvement 3.52 points (1.33)	Worsening 4.88 points (2.94)	8.40 points (3.23)	.027
Withdrawal	Improvement 1.24 points (2.20)	Worsening 13.88 points (3.98)	15.11 points (4.55)	.006
Adaptability	Improvement 3.19 points (1.61)	Worsening 7.13 points (2.98)	10.32 points (3.39)	.011
Social skills	Improvement 3.86 points (1.49)	Worsening 4.13 points (2.81)	7.98 points (3.18)	.029
ADHD probability index	Improvement 5.18 points (2.40)	Worsening 1.86 points (2.15)	7.02 points (3.23)	.042
Functional impairment index	Improvement 2.95 points (2.18)	Worsening 6.88 points (2.51)	9.83 points (3.32)	.009
Tremor	Improvement 0.38 points	Worsening 0.06 points	0.44 points (0.20)	.043
Cognition	Improvement 7.83 points (2.70)	Worsening 0.167 points (2.33)	7.99 points (3.57)	.032
9HPT	Improvement 0.67 points (2.02)	Worsening 2.92 points (2.25)	3.58 points (3.03)	.247
Standing balance	Improvement 4.29 points (2.06)	Improvement 5.18 points (3.07)	−0.90 points (3.70)	.811
OWLS-OE	Improvement 9.46 points (2.70)	Improvement 10.25 points (3.28)	−0.79 points (4.25)	.8536
OWLS-LC	Improvement 1.33 points (2.38)	Improvement 2.33 points (3.54)	−1.00 points (4.27)	.817

9HPT, 9-Hole-Pegboard Test; ADHD, attention deficit hyperactivity disorder; OWLS-LC, Oral and Written Language Scales-Listening Comprehension; OWLS-OE, Oral and Written Language Scales-Oral Expression; SE, standard error.

minimally clinically impactful change (MCIC) for each of the endpoints.

Safety

All doses of govorestat were generally well tolerated. There were no serious adverse events (SAEs) or deaths in the study. All TEAEs were mild or moderate with no severe TEAE reported. All (100%) participants on placebo treatment reported at least 1 event, and nearly all (96.8%) of the participants in the govorestat group reported at least one event. TEAEs were generally balanced between treatment groups and consistent with a pediatric study population (Table 3).

There were no serious or severe adverse events. In the placebo group, 12.5% of participants experienced a moderate TEAE versus 16.1% in the govorestat group. For most participants (87.5% of participants in the placebo group vs 80.6% in the govorestat group), TEAEs were mild. In the placebo group, 43.8% of participants had TEAEs that were considered related to the study drug versus 51.6% in the govorestat group. There were 6.3% of participants in the placebo group versus 19.4% in the govorestat group who reported TEAEs leading to interruption of the study drug. Two (6.5%) participants had non-serious and non-severe adverse events that led to permanent discontinuation in the govorestat group versus one (6.3%) participant in the placebo group. Permanent discontinuations were related to moderate alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) liver enzyme elevations, not associated with other liver function abnormalities, and never associated with an increase in bilirubin levels (no cases of Hy's law). All events leading to permanent discontinuation were asymptomatic and reversible within days after study drug discontinuation.

The most frequently reported TEAEs were gastrointestinal disorders (68.8% of participants in the placebo group and 74.2% in the govorestat group). Gastrointestinal TEAEs included vomiting (placebo: 31.3%; govorestat: 48.4%) and diarrhea (placebo: 12.5%; govorestat: 25.8%).

Modest changes in ALT and/or AST were observed in the placebo (12.5% of participants) and govorestat groups (25.8%). Pre-specified criteria for study drug interruption due to ALT and/or AST elevations were included in the study protocol. Six cases met these criteria (five in the govorestat-treated group and one

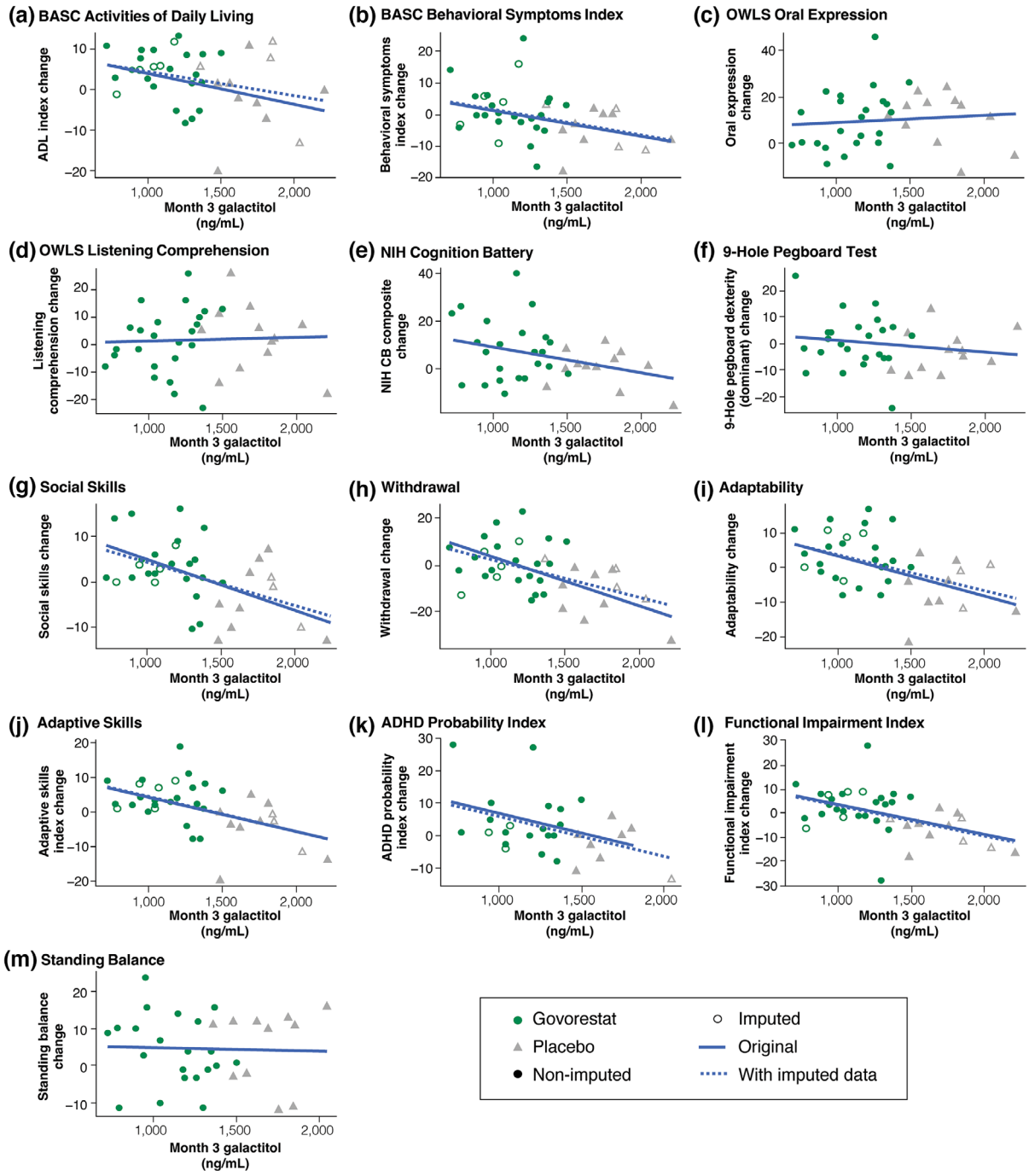

Figure 4. Pearson correlations showing galactitol level (ng/mL) at Month 3 compared with change in clinical outcomes from baseline to 18 months. (a) Behavior Assessment System for Children Activities of Daily Living (BASC-ADL). (s) Behavior Assessment System for Children Behavioral Symptoms Index (BASC-BSI). (c) Oral and Written Language Scales-Oral Expression (OWLS-OE). (d) Oral and Written Language Scales-Listening Comprehension (OWLS-LC). (e) National Institute of Health Cognition Batter (NIH-CB). (f) 9-Hole-Pegboard Test (9HPT). (g) BASC social skills. (h) BASC withdrawal. (i) BASC adaptability. (j) BASC adaptive skills. (k) BASC attention deficit hyperactivity disorder (ADHD) probability index. (l) BASC functional impairment index. (m) NIH standing balance test.

in placebo); three of the participants (all on govorestat) were able to restart treatment without further increases in ALT and/or AST. All cases were evaluated by an independent Hepatic Safety Adjudication Committee. ALT and/or AST elevations were not associated with increases in bilirubin or other liver enzymes, and all were asymptomatic. There were no cases of Hy's law.

Modest changes in renal function occurred both in the placebo and govorestat groups. Increased UACR

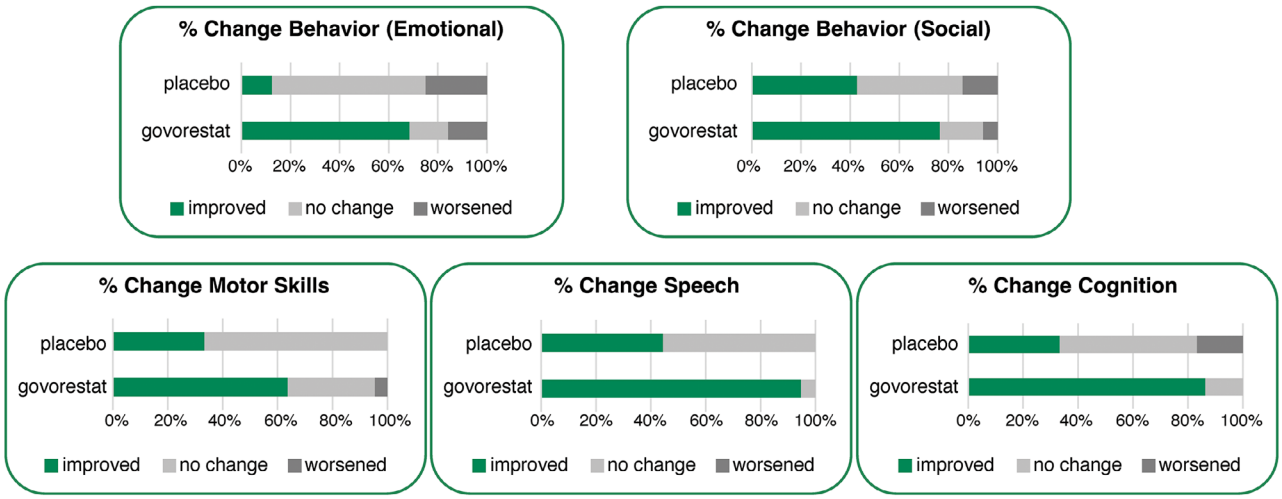

Figure 5. Exit interview results. Results shown as improvement (green), no change (light grey), or worsening (dark grey) from the caregiver perspective. All results are shown as percentages of participants in the treatment group.

Table 3. TEAEs in Govorestat and Placebo-Treated Groups

System Organ Class	Placebo Number (%) of Subjects	Govorestat Number (%) of Subjects
Gastrointestinal disorders	11 (68.8%)	23 (74.2%)
Vomiting	5 (31.3%)	15 (48.4%)
Diarrhea	2 (12.5%)	8 (25.8%)
Hepatic enzyme increased	2 (12.5%)	8 (25.8%)
Urine albumin/creatinine ratio increased	7 (43.8%)	5 (16.1%)
Urine protein/creatinine ratio increased	3 (18.8%)	2 (6.5%)
Infections and infestations	10 (62.5%)	18 (58.1%)
Viral upper respiratory tract infection	3 (18.8%)	14 (45.2%)

was observed in 43.8% of participants in the placebo group and 16.1% in the govorestat group along with increased UPCR observed in 18.8% of participants in the placebo group versus 6.5% in the govorestat group.

Viral upper respiratory tract infections affected 18.8% of participants in the placebo group and 45.2% of the govorestat group; none were considered related to the study drug in either group. Pyrexia occurred in 56.3% of participants in the placebo group versus 54.8% in the govorestat group with no event of pyrexia in either group considered as related to treatment. There were no allergic reactions. Skin and subcutaneous tissue disorders (dermatitis and urticaria) were reported in 6.3% of participants in the placebo group and 3.2% in the govorestat group and no event of dermatitis or urticaria was drug related in either group.

Discussion

The ACTION-Galactosemia Kids study was the first longitudinal study ever performed in children with Classic Galactosemia in which the same participants performed the same standardized tests repeatedly over time. The placebo group in this study provides the first longitudinal natural history data in this patient population, which on its own is vital information for better understanding this disease. The placebo group demonstrated a steady worsening from baseline on age-standardized tests of behavior, daily living skills, cognition, fine motor skills, and tremor, resulting in a widening gap between the children with Classic Galactosemia and their peers. Interestingly, speech and gross motor skills outcomes did not worsen over time in the placebo group, and many children in the study reported implementation of speech therapy and/or occupational therapy during the trial, suggesting that these interventions provide benefit, at least over an 18-month period of time.

Treatment with govorestat reduced galactitol levels and provided clinical benefit in the form of stabilization of clinical outcomes (preventing worsening) or improving outcomes on measures of behavioral symptoms, daily living skills, adaptive skills, cognition, fine motor skills, and tremor. On many of these outcomes, the difference between the govorestat group and the placebo group was an entire standard deviation on the T-scale or standard scale, which underscores the magnitude of the treatment effect. Additionally, in support of the clinical meaningfulness of these numerical changes in clinical outcomes, parents of children in the govorestat group reported improvement or stabilization compared with parents of children in the placebo group. Also, study exit interviews reflected that these changes were meaningful to the parents on a daily basis.

The placebo group in this study provides the first longitudinal data using standardized tests of behavior,

function, and cognition in children with Classic Galactosemia. Results indicate that the disease is progressive with children losing skills over time with regard to behavior and daily function as shown by both raw scores and standard scores declining over time. On cognition, children developed additional skills over time, but not at the rate of their normative peers, resulting in raw scores that remained the same, but standard scores that declined. In both cases, the result is a widening gap between children with Classic Galactosemia and their normative peers.

Galactitol level at an early timepoint (3 months) correlated with change in clinical outcomes at a later timepoint (18 months) in both the active and placebo groups providing strong mechanistic evidence that galactitol is driving changes in clinical outcomes over time. This result supports the study hypothesis that reduction in galactitol levels through aldose reductase inhibition improves outcomes in children with Classic Galactosemia.

Govorestat treatment led to a rapid and sustained reduction in plasma galactitol level without any compensatory increase in galactose or Gal-1p over time. Govorestat was generally safe and well tolerated with no serious safety signals reported. Asymptomatic (detected only by monitoring laboratory tests) and reversible ALT and AST elevations were detected at a slightly higher rate in govorestat-treated participants compared to placebo-treated participants. Participants were often able to restart treatment without a relapse in ALT/AST elevation. Many of these participants had experienced acute hepatic insult in the perinatal period due to dietary galactose exposure. Prior medical history of acute hepatic insult in the perinatal period may be a risk factor to explore in future work to determine whether this is predictive of future hepatic abnormalities.

Conclusions

Taken together, the progressive worsening over time in the placebo group compared to the stabilization or improvement in outcomes in the govorestat group suggests an urgent need for treatment and potential benefit of govorestat treatment for children with Classic Galactosemia. A longer treatment duration or a study that potentially controls for use of speech therapy and occupational therapy in children may be helpful in exploring clinical benefit of govorestat on speech and gross motor skills.

Govorestat treatment has been shown to reduce galactitol levels in adults with Classic Galactosemia; however, clinical outcomes have not yet been studied in adults. Future studies may seek to explore the benefit of galactitol reduction on clinical outcomes in adults with Classic Galactosemia.

Author Contributions

Riccardo Perfetti, Evan Bailey, Stella Wang, and Shoshana Shendelman designed the study and interpreted the data. Han Phan, Ayesha Ahmad, Janet Thomas, Elizabeth G. Ames, Amanda B. Pritchard, and Shane C. Quinonez performed the study at the investigative sites. Caleb Dayley, Andrew Salt, Christina Pick, Abe Durrant, Samuel Johnson, Jessie Nicodemus-Johnson, Samuel P. Dickson, and Suzanne B. Hendrix performed the statistical analyses and modeling. Riccardo Perfetti and Evan Bailey wrote the manuscript. All authors critically reviewed the manuscript and approved the final version for publication.

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Conflicts of Interest

Riccardo Perfetti, Evan Bailey, Stella Wang, and Shoshana Shendelman are employees and shareholders of Applied Therapeutics Inc. The authors affiliated with the study sites were paid by Applied Therapeutics Inc. to conduct the study. The authors affiliated with Pentara Corporation were paid consultants of Applied Therapeutics Inc.

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Data Availability Statement

The pharmacokinetic, pharmacodynamic, safety, and clinic outcome source data used in this study were made available to Pentara Corporation for an third party data analysis. The data that support the findings of this study are not available due to legal restrictions.

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